Protocol for purification of recombinant proteins

E. coli strain BL21(DE3) harboring the appropriate plasmid was grown at 37 °C in 2xYT medium overnight with suitable concentration of antibiotic. The culture was diluted 100 fold into fresh medium with antibiotic and grown at 37°C to an optical density of 0.6~0.8 at 600 nm, the protein expression was induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and cells were grown overnight at 25°C.

Cells were centrifuged at 8000rpm for 15min at 4°C. Resuspend the cell paste expressing recombinant protein in binding buffer (20 mM Tris-HCl, 0.5 M NaCl, 20 mM imidazole, 1mM β-mercaptoethanol, pH7.4), containing *SIGMAFAST*™ Protease Inhibitor Cocktail Tablets (SIGMA-ALORICH®). Disrupt the cells with sonication for 20 min with suitable power on ice and centrifuge at 18000 rpm for 40 min at 4°C. Remove remaining particles by passing the supernatant through a 0.22 μm filter.

The HisTrap™ column (GE Healthcare, Inc.) was equilibrated with binding buffer. Load the sample and wash the column with binding buffer.

Elute the target protein with a linear gradient starting with binding buffer and ending with the same buffer including 500mM imidazole. The eluted fraction

containing the target protein were concentrated by Amicon® Ultra Centrifugal Filters (Merck) with a 10 kDa cutoff, then frozen by liquid nitrogen and stored at -80°C.

Protein purification was checked by SDS-PAGE and the resulting protein is quantified by Braford analysis.